Karolinska Institutet

Department of Cell and Molecular Biology

EPSTEIN-BARR VIRUS AND GENOMIC INSTABILITY- A NEW LOOK AT THE MECHANISMS OF VIRAL ONCOGENESIS

AKADEMISK AVHANDLING
Som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i CMB föreläsningssal, Berzelius väg 21

Onsdag den 12 January 2011, klockan 09:30

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Stockholm 2011

ABSTRACT

EBV is associated with a variety of lymphoid and epithelial malignancies but the mechanisms of oncogenesis are still not fully understood. The aim of the work described in this thesis was to assess whether induction of genomic instability, as defined by the accumulation of non-clonal genetic aberrations, could play a role in EBV oncogenesis and identify the viral protein(s) responsible for this phenotype. Cytogenetic analysis of a panel of EBV(+) and EBV(–) Burkitt`s lymphoma cell lines revealed a significant increase in dicentric chromosomes, chromosome fragments and chromatid gaps in EBV infected cells. EBV latency I, where only EBNA1 is expressed, was sufficient for this effect, whereas a stronger increase was observed in latency III suggesting the involvement of several latency proteins. Telomere analysis by fluorescent in situ hybridization (FISH) showed an increase in the prevalence of telomere fusion and double strand break fusion in dicentric chromosomes from EBV(+) cells pointing to telomere dysfunction and DNA double strand breaks (DSBs) as possible mechanisms by which EBV may promote genomic instability.

The significant increase of chromosomal aberrations in cells expressing latency I suggests a possible role for EBNA1 in the induction of genomic instability. This was confirmed by analyzing the occurrence of chromosomal aberrations, DSBs and engagement of DNA damage response (DDR) in B-cell lymphoma cell lines expressing constitutive or inducible EBNA1. EBNA1 expression correlated with a significant increase of reactive oxygen species (ROS) suggesting a possible role for oxidative stress, which was confirmed by the decrease of chromosome abnormalities in cells treated with ROS scavengers. EBNA1 was then shown to induce oxidative stress by transcriptional activation of the catalytic subunit of NADPH oxidise, NOX2.

Stable or conditional expression of EBNA1 was associated with the accumulation of telomere abnormalities, including loss and gain of telomere signals, telomere fusion and heterogeneous length of telomeres. This phenotype was coupled with the accumulation of extra-chromosomal telomeres, telomere dysfunction induced foci (TIFs), telomere-associated promyelocytic leukemia nuclear bodies (T-PNBs) and telomere-sister chromatid exchanges (T-SCEs), and with displacement of the shelterin protein TRF2 from telomeres. The induction of TIFs and T-PNBs was inhibited by treatment with scavengers of ROS that also promoted the re-localization of TRF2 at telomeres.

EBNA1 regulates virus replication and transcription, and participates in the remodeling of the cellular environment that accompanies EBV induced B-cell immortalization. We have profiled the transcriptional changes induced by short- and long-term expression of EBNA1 in the EBV negative B-cell lymphoma BJAB. Gene ontology analysis of forty seven genes that were consistently regulated independently on the time of EBNA1 expression revealed an unexpected enrichment of genes involved in the maintenance of chromatin architecture. The protein interaction network of the affected gene products suggests that EBNA1 may promote a broad rearrangement of the cellular transcription landscape by altering the expression of key components of chromatin remodeling complexes.

Collectively these studies highlight previously unrecognized mechanisms by which EBNA1 may promote malignant transformation and tumor progression through induction of oxidative stress and by promoting the epigenetic reprogramming of EBV infected cells.

Key Words: EBV, EBNA1, ROS, TIFs, T-PNBs, Burkitt`s Lymphoma, T-SCEs

ISBN 978-91-7457-187-5